

HYDROXY-AMINO ACID AMIDES

FIELD OF THE INVENTION

The present invention relates to amino acid (statine) analogs that display selective inhibitory activity against plasmepsin and cathepsin D.

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BACKGROUND OF THE INVENTION

Resistance to known antimalarial therapies is becoming an increasing problem and new therapies are therefore desperately needed. Upon infecting a host, the malaria parasite avidly consumes the host hemoglobin as its source of nutrients. Plasmepsin I and II are proteases from *Plasmodium falciparum* that are necessary during the initial stages of hemoglobin hydrolysis and digestion, which primarily occurs in the α -chain, between Phe 33 and Leu 34, although other sites may serve as substrates for hydrolysis as well. It has been shown in cultures inhibition of plasmepsin by a peptidomimetic inhibitor is effective in preventing malarial hemoglobin degradation and in killing the parasite (Francis, S.E., Gluzman, I.Y. Oksman, A., Knickerbocker, A., Mueller, Bryant, M.L., Sherman, D.R., Russell, D.G., and Goldberg, D.E. (1994) *EMBO J.*, **13**, 306-317). Thus, persons of skill in the art expect that plasmepsin inhibitors will provide effective antimalarial therapy.

Cathepsin D is a human protease in the endosomal-lysosomal pathway,

involved in lysosomal biogenesis and protein targeting, and may also be involved in

antigen processing and presentation of peptide fragments. The protease therefore displays broad substrate specificity but prefers hydrophobic residues on either side of the scissile bond.

5 Cathepsin D has been implicated in a variety of diseases, including connective tissue disease, muscular dystrophy, and breast cancer. Most recently, cathepsin D is believed to be γ -secretase, the protease which processes the β -amyloid precursor protein to generate the C-terminus of β -amyloid (Dreyer, R.N., Bausch, K.M., Fracasso, P., Hammond, L.J., Wunderlich, D., Wirak, D.O., Davis, G., Brini, C.M., Bucholz, T.M., Konig, G., Kamark, M.E., and Tamburini, P.P. (1994) Eur. J. Biochem., 224, 265-271 and Ladror, U.S., Synder, S.W., Wang, G.T., Holzman, and Krafft, G.A. (1994) J. Biol. Chem., 269, 18422-18428), which is the major component 10 of plaque in the brains of Alzheimer's patients. Consequently, persons of skill in the art expect that inhibitors of cathepsin D will be useful in treating Alzheimer's disease.

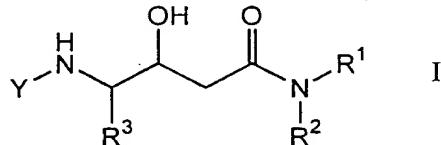
15 The present invention relates to amino acid (statine) analogs and their inhibitory action against aspartyl proteases, and more particularly, the invention relates to the identification of amino acid analogs that display selective inhibitory 20 activity against plasmeprin and cathepsin D. Although statine-containing peptides are known which inhibit aspartyl proteases (Shewale, J. G.; Takahashi, R.; Tang, J. , *Aspartic Proteinases and Their Inhibitors*, Kostka, V., Ed. Wlater de Gruyter: Berlin (1986) pp 101-116), there are only a few selective inhibitors for cathepsin D (Lin, T.-

Y.; Williams, H. R., *Inhibition of Cathepsin D by Synthetic Oligopeptides*, *J. Biol. Chem.* (1979), 254, 11875-11883; Rich, D. H.; Agarwal, N. S., *Inhibition of Cathepsin D by Substrate Analogues Containing Statine and by Analogues of Pepstatin*, *J. Med. Chem.* (1986) 29 (2519-2524), and for plasmeprin (Silva, A. M. et al., *Structure and Inhibition of Plasmeprin II, A Hemoglobin-Degrading Enzyme From Plasmodium falciparum*, *Proceed Natl Acad Sci*, 1996, 93, 10034-10039). The present invention also relates to the solid phase synthesis of such amino acid analogs.

SUMMARY OF THE INVENTION

I. Preferred Embodiments

The compounds of the present invention are represented by Formula I:



wherein:

R¹ and R³ are independently chosen from the group consisting of alkyl, alkoxyalkyl and arylalkyl;

R² is H or (S)-C(O)-L-

wherein :

(S) is a solid support; and

-L- is a linker; and

Y is -Aa-C(O)R⁴ or -C(O)R⁵;

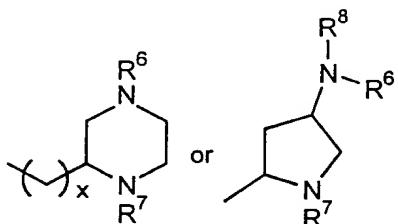
wherein

Aa is an amino acid attached via its carboxyl to the amine nitrogen of structure I;

R⁴ is chosen from the group consisting of alkyl, aryl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl or substituted heterocycloalkyl; and

T50X

5 R⁵ is



wherein

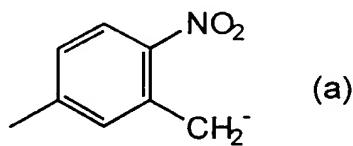
x is 0 or 1;

R⁶ and R⁷ are independently chosen from the group consisting of substituted alkyl, alkylcarbonyl and substituted alkylcarbonyl; and

10 R⁸ is alkyl.

Preferred compounds of Formula I are those wherein -L- is of Formula (a)

T51X



wherein the left-hand bond is the point of attachment to C(O) and the right hand bond is the point of attachment to the amide nitrogen of structure I.

15 A preferred embodiment of the invention are compounds of Formula I

wherein:

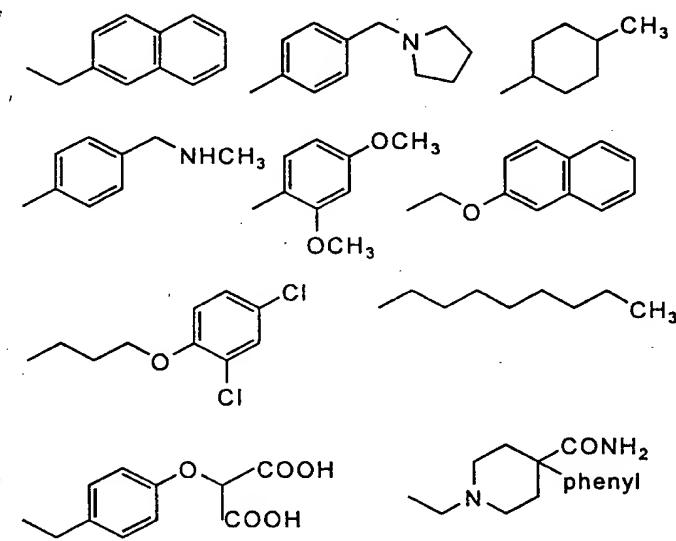
R^1 is chosen from the group consisting of butyl, 3-phenylpropyl and 3-methoxypropyl;

Y is $-Aa-C(O)R^4$;

5 Aa is chosen from the group consisting of valine, leucine, phenylalanine,

isoleucine
 α ~~2-amino-3,4-dimethylpentanoic acid~~, β -2-thienylalanine, t-butylglycine, cysteine and phenylglycine; and

R^4 is chosen from the group consisting of



Another preferred embodiment of the invention are compounds of Formula I
wherein:

R¹ is chosen from the group consisting of methyl, benzyl, butyl, 3-phenylpropyl,
3-methoxypropyl, 2-pyridinylmethyl and 3-pyridinylmethyl;

5 Y is -C(O)R⁵;

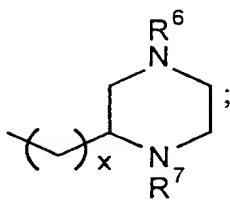
R⁶ is chosen from the group consisting of 3-pyridinylmethyl, phenylethoxyethyl,
3,4,5-trimethoxybenzyl, 4-acetamidobenzyl, 4-phenylbutyl, 3,4-
dichlorobenzyl, 4-phenylbenzyl, 3-phenylpropyl, 3,5-
bis(trifluoromethyl)benzyl, 3-phenylpropionyl, isobutyl, propionyl and 3,5-
di(trifluoromethyl)phenylacetyl; and

R⁷ is chosen from the group consisting of 4-isopropoxybenzoyl, nicotinoyl,
3,4,5-trimethoxybenzoyl, 3-phenoxybenzoyl, 3-(2-methoxyphenyl)propyl,
3,4,5-trimethoxyphenylpropionyl, 3,3-diphenylpropionyl, phenylacetyl, 3,4-
dichlorophenylacetyl and ethyl adipoyl.

15 A preferred subset of the foregoing embodiment of the invention are
compounds of the Formula I wherein:

R¹ is chosen from the group consisting of methyl, benzyl, butyl, 3-phenylpropyl
and
3-methoxypropyl;

20 Y is -C(O)R⁵;

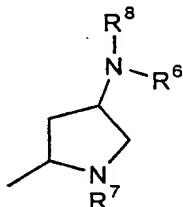
*T80X**R⁵ is*

R⁶ is chosen from the group consisting of 3-pyridinylmethyl, phenylethoxyethyl, 3,4,5-trimethoxybenzyl, 4-acetamidobenzyl, 4-phenylbutyl, 3,4-dichlorobenzyl, 4-phenylbenzyl, 3,5-bis(trifluoromethyl)benzyl, 3-phenylpropionyl and 3-phenylpropyl; and

R⁷ is chosen from the group consisting of 4-isopropoxybenzoyl, nicotinoyl, 3,4,5-trimethoxybenzoyl, 3-phenoxybenzoyl, 3-(2-methoxyphenyl)propyl, 3,4,5-trimethoxyphenylpropionyl, 3,3-diphenylpropionyl, 3,4-dichlorophenylacetyl and ethyl adipoyl.

A second subset of the second preferred embodiment of the invention are compounds of Formula I wherein

R¹ is chosen from the group consisting of butyl, 2-pyridinylmethyl and 3-pyridinylmethyl;

R⁵ is

R⁶ is chosen from the group consisting of 4-phenylbenzyl, isobutyl, propionyl and 3,5-di(trifluoromethyl)phenylacetyl;

R⁷ is chosen from the group consisting of phenylacetyl, 3-phenoxybenzoyl and 3,3-diphenylpropionyl; and

R⁸ is ethyl.

Another aspect of the invention is the use of divinylbenzene-cross-linked, 5 polyethyleneglycol-grafted polystyrene beads optionally functionalized with amino groups (e.g., TentaGel™ S NH₂, Rapp Polymere) as the solid supports for constructing compounds of Formula I.

DETAILED DESCRIPTION OF THE INVENTION

II. Abbreviations and Definitions

The following abbreviations and terms have the indicated meaning throughout:

Alloc	=	allyloxy carbonyl
Bn	=	benzyl
BNB	=	4-bromomethyl-3-nitrobenzoic acid
BOC	=	t-butyloxy carbonyl
15 Bu	=	butyl
c-	=	cyclo
DCM	=	Dichloromethane = methylene chloride = CH ₂ Cl ₂
DIC	=	diisopropylcarbodiimide
DIEA	=	diisopropylethyl amine
20 DMAP	=	4-N,N-dimethylaminopyridine
DMF	=	N,N-dimethylformamide
DVB	=	1,4-divinylbenzene

Et	=	ethyl
Fmoc	=	9-fluorenylmethoxycarbonyl
HATU	=	O-(7-Azabenzotriazol-1-yl)1,1,3,3-tetramethyluronium- hexafluorophosphate
5	HOAc	= acetic acid
	HOBt	= hydroxybenzotriazole
	m-	= meta
	Me	= methyl
	N ₃	= azido
10	NaBH ₃ CN	= sodium cyanoborohydride = SCB
	PEG	= polyethylene glycol
	Ph	= phenyl
	s-	= secondary
	t-	= tertiary
15	TFA	= trifluoroacetic acid
	THF	= tetrahydrofuran

“Alkyl” is intended to include linear, branched, or cyclic hydrocarbon

structures and combinations thereof. “Lower alkyl” means alkyl groups of from 1 to 8

carbon atoms. Examples of lower alkyl groups include methyl, ethyl, propyl,

20 isopropyl, butyl,

s-and t-butyl, pentyl, hexyl, octyl, cyclopropylenthyl, bornyl and the like. Preferred

alkyl groups are those of C₂₀ or below.

“Cycloalkyl” is a subset of alkyl and includes cyclic hydrocarbon groups of from 3 to 8 carbon atoms. Examples of lower cycloalkyl groups include c-propyl, c-butyl,
5 c-pentyl, norbornyl and the like.

“Alkenyl” includes C₂-C₈ unsaturated hydrocarbons of a linear, branched, or cyclic (C₅-C₆) configuration and combinations thereof. Examples of alkenyl groups include vinyl, allyl, isopropenyl, pentenyl, hexenyl, c-hexenyl, 1-propenyl, 2-butenyl, 2-methyl-2-butenyl and the like.

10 “Alkynyl” includes C₂-C₈ hydrocarbons of a linear or branched configuration and combinations thereof containing at least one carbon-carbon triple bond. Examples of alkynyl groups include ethyne, propyne, butyne, pentyne, 3-methyl-1-butyne, 3,3-dimethyl-1-butyne and the like.

15 “Alkoxy” refers to groups of from 1 to 8 carbon atoms of a straight, branched, cyclic configuration and combinations thereof. Examples include methoxy, ethoxy, propoxy, isopropoxy, cyclopropoxy, cyclohexyloxy and the like.

“Acylamino” refers to acylamino groups of from 1 to 8 carbon atoms of a

straight, branched or cyclic configuration and combinations thereof. Examples include acetylamino, butylamino, cyclohexylamino and the like.

"Halogen" includes F, Cl, Br, and I.

"Aryl" and "heteroaryl" mean a 5- or 6-membered aromatic or heteroaromatic ring containing 0-3 heteroatoms selected from O, N, and S; a bicyclic 9- or 10-membered aromatic or heteroaromatic ring system containing 0-3 heteroatoms selected from O, N, and S; or a tricyclic 13- or 14-membered aromatic or heteroaromatic ring system containing 0-3 heteroatoms selected from O, N, and S; each of which rings is optionally substituted with 1-3 lower alkyl, substituted alkyl, substituted alkynyl, =O, -NO₂, halogen, hydroxy, alkoxy, OCH(COOH)₂, cyano, NR¹⁰R¹⁰, acylamino, phenyl, benzyl, phenoxy, benzyloxy, heteroaryl, and heteroaryl or heteroaryloxy; each of said phenyl, benzyl, phenoxy, benzyloxy, heteroaryl, and heteroaryloxy is optionally substituted with 1-3 substituents selected from lower alkyl, alkenyl, alkynyl, halogen, hydroxy, alkoxy, cyano, phenyl, benzyl, benzyloxy, carboxamido, heteroaryl, heteroaryloxy, NO₂, and NR¹⁰R¹⁰;

R¹⁰ is independently H, lower alkyl or cycloalkyl, and -R¹⁰R¹⁰ may be fused to form a cyclic ring with nitrogen.

The aromatic 6- to 14-membered carbocyclic rings include, e.g., benzene,

naphthalene, indane, tetralin, and fluorene and the 5- to 10-membered aromatic heterocyclic rings include, *e.g.*, imidazole, pyridine, indole, thiophene, benzopyranone, thiazole, furan, benzimidazole, quinoline, isoquinoline, quinoxaline, pyrimidine, pyrazine, tetrazole, and pyrazole.

5 "Arylalkyl" means an alkyl residue attached to an aryl ring. Examples

include, *e.g.*, benzyl, phenethyl and the like.

"Heteroarylalkyl" means an alkyl residue attached to a heteroaryl ring.

Examples include, *e.g.*, pyridinylmethyl, pyrimidinylethyl and the like.

10 "Heterocycloalkyl" means a cycloalkyl where one to two of the methylene (CH_2) groups is replaced by a heteroatom such as O, NR' (wherein R' is H or alkyl), S or the like; with the proviso that when two heteroatoms are present, they must be separated by at least two carbon atoms. Examples of heterocycloalkyls include tetrahydrofuranyl, piperidine, dioxanyl and the like.

15 "Carboxyalkyl" means $-\text{C}(\text{O})\text{R}'$, wherein R' is alkyl.

"Substituted" alkyl, alkenyl, alkynyl, cycloalkyl, or heterocycloalkyl means alkyl, alkenyl, alkynyl, cycloalkyl or heterocycloalkyl wherein up to three H atoms on each C atom therein are replaced with halogen, hydroxy, loweralkoxy, carboxy,

carboalkoxy, carboxamido, cyano, carbonyl, NO₂, NR⁹R⁹ (wherein R⁹ is H, alkyl or arylalkyl), alkylthio, sulfoxide, sulfone, acylamino, amidino, phenyl, benzyl, heteroaryl, phenoxy, benzyloxy, heteroaryloxy, and substituted phenyl, benzyl, heteroaryl, phenoxy, benzyloxy or heteroaryloxy.

5 Aa represents an amino acid and is intended to include the racemates and all optical isomers thereof. The amino acid side chains of Aa include, *e.g.*, methyl (alanine), hydroxymethyl (serine), phenylmethyl (phenylalanine), thiomethyl (cysteine), carboxyethyl (glutamic acid), *etc.* Primary and secondary amino acids are intended to include alanine, asparagine, N- β -trityl-asparagine, aspartic acid, aspartic acid- β -t-butyl ester, arginine, N^g-Mtr-arginine, cysteine, S-trityl-cysteine, glutamic acid, glutamic acid- γ -t-butyl ester, glutamine, N- γ -trityl-glutamine, glycine, histidine, N^{im}-trityl-histidine, isoleucine, leucine, lysine, N^e-Boc-lysine, methionine, phenylalanine, proline, serine, O-t-butyl-serine, threonine, tryptophan, Nⁱⁿ-Boc-tryptophan, tyrosine, valine, 15 sarcosine, L-alanine, chloro-L-alanine, 2-aminoisobutyric acid, 2-(methylamino)isobutyric acid, D, L-3-aminoisobutyric acid, (R)-(-)-2-aminoisobutyric acid, (S)-(+)-2-aminoisobutyric acid, 2-thienylalanine, D-norvaline, L-norvaline, L-2-amino-4-pentenoic acid, 20 D-isoleucine, L-isoleucine, D-norleucine, 2,3-diaminopropionic acid, L-norleucine, D,L-2-aminocaprylic acid β -alanine, D,L-3-aminobutyric acid, 4-aminobutyric acid,

4-(methylamino)butyric acid, 5-aminovaleric acid, 5-aminocaproic acid,
7-aminoheptanoic acid, 8-aminocaprylic acid, 11-aminodecanoic acid,
12-aminododecanoic acid, carboxymethoxylamine, D-serine, D-homoserine,
L-homoserine, D-allothreonine, L-allothreonine, D-threonine, L-threonine,
5 D,L-4-amino-3-hydroxybutyric acid, D,L-3-hydroxynorvaline, (3S,4S)-(-)-statine,
5-hydroxy-D,L-lysine, 1-amino-1-cyclopropanecarboxylic acid, 1-amino-1-
cyclopentanecarboxylic acid, 1-amino-1-cyclohexanecarboxylic acid,
5-amino-1,3-cyclohexadiene-1-carboxylic acid, 2-amino-2-norbornanecarboxylic
acid, (S)-(-)-2-azetidinecarboxylic acid, cis-4-hydroxy-D-proline, cis-4-hydroxy-L-
10 proline, trans-4-hydroxy-L-proline, 3,4-dehydro-D,L-proline, 3,4-dehydro-L-proline,
pipecolic acid, pipecolinic acid, nipecotic acid, isonipecotic acid, mimosine, citrulline,
2,3-diaminopropionic acid, D,L-2,4-diaminobutyric acid, (S)-(+)-diaminobutyric acid,
15 ornithine, 2-methylornithine, N- ϵ -methyl-L-lysine, N-methyl-D-aspartic acid, D,L-2-
methylglutamic, D,L-2-amino adipic acid, D-2-amino adipic acid, naphthylalanine,
L-2-amino adipic acid, (+/-)-3-amino adipic acid, D-cysteine, D-penicillamine,
L-penicillamine, D,L-homocysteine, S-methyl-L-cysteine, L-methionine, D-
ethionine,
L-ethionine, S-carboxymethyl-L-cysteine, (S)-(+)-2-phenylglycine,
20 (R)-(-)-2-phenylglycine, N-phenylglycine, N-(4-hydroxyphenyl)glycine,
D-phenylalanine, (S)-(-)-indoline-2-carboxylic acid, α -methyl,D,L-phenylalanine,
 β -methyl-D,L-phenylalanine, D-homophenylalanine, L-homophenylalanine,
D,L-2-fluorophenylglycine, D,L-2-fluorophenylalanine, D,L-3-fluorophenylalanine,

D,L-4-fluorophenylalanine, D,L-4-chlorophenylalanine, L-4-chlorophenylalanine,
4-bromo-D,L-phenylalanine, 4-iodo-D-phenylalanine, 3,3',5-triiodo-L-thyronine,
(+)-3,3',5-triiodo-L-thyronine sodium salt, D-thyronine, L-thyronine, D,L-m-tyrosine,
D-4-hydroxyphenylglycine, D-tyrosine, L-tyrosine, o-methyl-L-tyrosine, 3-fluoro-
5 D,L-tyrosine, 3-iodo-L-tyrosine, 3-nitro-L-tyrosine, 3,5-diodo-L-tyrosine, D,L-dopa,
L-dopa, 2,4,5-trihydroxyphenyl-D,L-alanine, 3-amino-L-tyrosine, 4-amino-D-
phenylalanine,
4-amino-L-phenylalanine, 4-amino-D,L-phenylalanine, 4-nitro-L-phenylalanine,
4-nitro-D,L-phenylalanine, 3,5-dinitro-L-tyrosine, D,L- α -methyltyrosine,
L- α -methyltyrosine, (-)-3-(3,4-dihydroxyphenyl)-2-methyl-L-alanine,
D,L-threo-3-phenylserine, trans-4-(aminomethyl)cyclohexane carboxylic acid, 4-
(aminomethyl)benzoic acid, D,L-3-aminobutyric acid, 3- aminocyclohexane
carboxylic acid, cis-2-amino-1-cyclohexane carboxylic acid, γ -amino- β -(p-
chlorophenyl) butyric acid (Baclofen), D,L-3-aminophenylpropionic acid, 3-amino-3-
15 (4-chlorophenyl) propionic acid, 3-amino-3-(2-nitrophenyl)propionic acid,
cyclohexylalanine, t-butylglycine, pyridylalanine and 3-amino-4,4,4-trifluorobutyric
acid.

The statine residues used in this invention were prepared by the method of
Rich (Rich *et al.*, J. Org. Chem., **43**, 3624 (1978)).

20 The material upon which the combinatorial syntheses of the invention are

performed are referred to as solid supports, beads or resins. These terms are intended to include beads, pellets, disks, fibers, gels, or particles such as cellulose beads, pore-glass beads, silica gels, polystyrene beads optionally cross-linked with divinylbenzene and optionally grafted with polyethylene glycol and optionally functionalized with 5 amino, hydroxy, carboxy, or halo groups, grafted co-poly beads, poly-acrylamide beads, latex beads, dimethylacrylamide beads optionally cross-linked with N,N'-bis-acryloyl ethylene diamine, glass particles coated with hydrophobic polymer, *etc.*, *i.e.*, material having a rigid or semi-rigid surface; and soluble supports such as low molecular weight non-cross-linked polystyrene.

10 **III. Optical Isomers - Diastereomers - Geometric Isomers**

Some of the compounds described herein contain one or more asymmetric centers and may thus give rise to enantiomers, diastereomers, and other stereoisometric forms which may be defined in terms of absolute stereochemistry as (R)- or (S)-, or as (D)- or (L)- for amino acids. The present invention is meant to include all such possible diastereomers, as well as their racemic and optically pure forms. Optically active (R)- and (S), or (D)- and (L)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the 15 compounds include both E and Z geometric isomers. Likewise, all tautomeric forms 20 are intended to be included.

IV. Assays for Determining Biological Activity**1. Method for Plasmeprin II**

The assay mix contained 50 mM sodium acetate (pH 5.0), 1 mg/ml BSA, 0.01% Tween 20, 12.5% glycerol, 18 % DMSO and 12 μ M plasmeprin substrate.

5 Twenty five μ L of the assay mix was added to each well of a 96-well microtiter plate containing dried down bead eluate or empty control wells. The plates were then sonicated and mixed. 25 μ L of 8 nM plasmeprin II, in 50 mM sodium acetate (pH 5.0), 1 mg/ml BSA, 0.01% Tween 20, and 12.5% glycerol, was added to the assay mix. The final concentrations were 4 nM plasmeprin II, 6 μ M plasmeprin substrate, 9 % DMSO, 50 mM sodium acetate (pH 5.0), 1 mg/ml BSA, 0.01% Tween 20, and 12.5% glycerol. The reaction was incubated for 10 minutes at 25 °C and then quenched by the addition of 25 μ L of 1 M Tris (pH 8.5) and 50 % DMSO to achieve a final concentration of 0.33 M Tris and 23% DMSO. The EDANS fluorescence was measured using a Tecan, SLT FluoStar fluorescence plate reader with an excitation filter of 350 nm and an emission filter 510 nm. The background was determined by 25 μ L of 50 mM sodium acetate (pH 5.0), 1 mg/ml BSA, 0.01% Tween 20, and 12.5% glycerol without enzyme.

2. Method for Cathepsin D

The assay mix contained 25 mM sodium formate (pH 3.5), 1 mg/ml BSA, 12 % DMSO and 12 μ M cathepsin D substrate. Twenty five μ L of the assay mix were added to each well of a 96-well microtiter plate containing dried down bead eluate or

empty control wells. The plates were then sonicated and mixed. 25 μ L of 1.6 nM cathepsin D, in 25 mM sodium formate (pH 3.5), and 1 mg/ml BSA, was added to the assay mix. The final concentrations were 0.8 nM cathepsin D, 6 μ M cathepsin D substrate, 6 % DMSO, 25 mM sodium formate (pH 3.5), and 1 mg/ml BSA. The reaction was incubated for 10 minutes at 25 °C and then quenched by the addition of 25 μ L of 1 M Tris (pH 8.5) and 50% DMSO to achieve a final concentration of 0.33 M Tris and 21 % DMSO. The EDANS fluorescence was measured as stated herein above. The background was determined by 25 μ L of 50 mM sodium formate (pH 3.5), and 1 mg/ml BSA without enzyme.

10 **V. Methods of Synthesis**

15 The compounds of the present invention may be prepared according to the following methods. In carrying out the syntheses, one typically begins with a quantity of solid support that will provide enough compound after cleavage from the solid support for biological testing in the herein described assays. In the case where the solid support is TentaGel™, it is recommended that approximately 0.5 g of beads of about 180 microns in diameter, with a loading capacity of about 300 picoM per bead, be used. As the chemical yield of compounds after photolysis typically ranges from approximately 20% up to 60%, this quantity will provide a yield (approximately >10 mg) sufficient for biological testing in the given protease assays. For actual synthesis, 20 the appropriate reagents and reaction conditions are applied to a reaction vessel containing the specified quantity of beads. During the syntheses, the beads may be

washed free of any excess reagents or by-products before proceeding to the next reaction. At the end of a given reaction sequence, the beads are suspended in a suitable solvent such as methanol and exposed to UV light (365 nm) for 3 hours at room temperature. This protocol releases the compounds of Formula I (wherein R² is H) for purification and biological testing.

5

A. Scheme 1: Derivatizing resin with bis-Boc lysine

A batch of amino-functionalized PEG-grafted polystyrene beads **3**, *e.g.*, TentaGel™ **3** amine may be modified with bis-Boc lysine **2** to increase the available reaction sites for ligand attachment. Bis-Boc lysine **2** is coupled to the amino-functionalized beads **3** by amide bond formation. Coupling is achieved by reacting a suspension of beads in DCM and adding **2**, HOBr and DIC. The suspension is shaken overnight, drained or filtered, and then washed in succession with DMF, MeOH and DCM, yielding derivatized resin **1** which is then dried overnight under vacuum.

B. Scheme 2

The various amine choices (see Tables 1 and 2) are added to the reaction vessel containing resin **1**. The amines are attached to resin **1** through the photo-labile linker,

4-bromomethyl-3-nitrobenzoic acid. This attachment is accomplished in two steps.

Step 1. The Boc protecting group on resin **1** is removed and the BNB is

attached by the following method. A suspension of resin **1** in 1:1 TFA/DCM is shaken for about 1 hour, then washed with DCM, MeOH, 4:1 MeOH/Et₃N, MeOH, DMF and then DCM. The resultant bis-amine resin **4** is suspended in DCM, and treated with a solution of BNB, HOBr and DIC in DCM. The suspension is shaken for about 3 hours, then drained and washed with DCM. The BNB resin **6** is dried overnight under vacuum.

Step 2. The BNB resin **6** from step 1 are reacted with a unique primary amine (see Tables 1 and 2) to generate compound **7**. The coupling of the amine to resin **6** occurs through displacement of the linker bromide and formation of a new carbon-nitrogen bond. As a quality control for the reaction in this step, a small portion of each batch of resin may be removed and titrated with picric acid to determine the extent of amine loading.

C. Scheme 3

Amine 7 is then treated with one of the hydroxy-amino acid reagents (statines) 8 (see Tables 1 and 2). Each hydroxy-amino acid 8 is coupled to amine resin 7 by amide bond formation to produce compound 9.

Compounds **9** may be directed through either the chemistry of Schemes 4 and 5, yielding compounds as in Example 1 and found in Table 1, or in the alternative, through the chemistry of Schemes 6, 7 and 8, yielding compounds as in Example 2

and Table 2.

5 D. Scheme 4

(With Scheme 5, yields compounds as in Example 1)

Resin **9** is treated with TFA/DCM to remove the Boc protecting group, thus exposing the terminal amino group and forming compounds **10**. Each reaction vessel is then treated with one amino acid **11** (see Table 1), for separate coupling of each amino acid to compound **10** by amide bond formation to produce compounds **12** . The amino acids are introduced with the base-labile Fmoc on the alpha-nitrogen atom.

10 E. Scheme 5

Compounds **12** are treated with piperidine/DMF to deprotect the amino group by removing the Fmoc protecting group, thus giving rise to compounds **13**, which in turn are treated with one carboxylic acid (see Table 1), which couples with compound **13** to generate compounds **14**. Resin **14** may be cleaved by exposing it to UV light (ca. 360 nm) for 15-180 minutes at 25-50 °C in a suitable solvent such as methanol to produce amides of Formula I (wherein R² is H), as in Example 1 and Table 1.

15 F. Scheme 6

(With Schemes 7 and 8, yields compounds as in Example 2)

Resin **9** (from Scheme 3) is treated with TFA/DCM to remove the Boc protecting group, thus exposing the terminal amino group and forming compounds **10**.

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The compound is then treated with one diamino acid **17** (Table 2), for separate coupling of each diamino acid **17** to compounds **10** by amide bond formation, using HATU and DIEA, to produce compounds **18**.

G. Scheme 7

5 Resin **18** is treated with TFA/DCM to selectively remove the Boc protecting group on the diamino acid ligand to produce amines **19**. The resin is then treated with one carboxylic acid reagent or one carboxyaldehyde (see Table 2) for either separate coupling of each carboxylic acid to compound **19** by amide bond formation or separate reductive amination of each carboxyaldehyde to compound **19** with sodium cyanoborohydride in methanol to produce resin **20**.

H. Scheme 8

Compounds **20** are then treated with palladium tetrakis(triphenylphosphine), tributyltin hydride in acetic acid and DCM to selectively remove the Alloc protecting group on the diamino acid ligand to produce amines **21**. Each vessel is then treated with one carboxylic acid reagent (see Table 2) for separate coupling of the carboxylic acid to compounds **21** by amide bond formation to produce resin **22**. Resin **22** may be cleaved by exposing the resin to UV light (*ca.* 360 nm) for 15-180 minutes at 25-50 °C in a suitable solvent such as methanol to produce amides Formula I (wherein R² is H), as in Example 2 and Table 2.

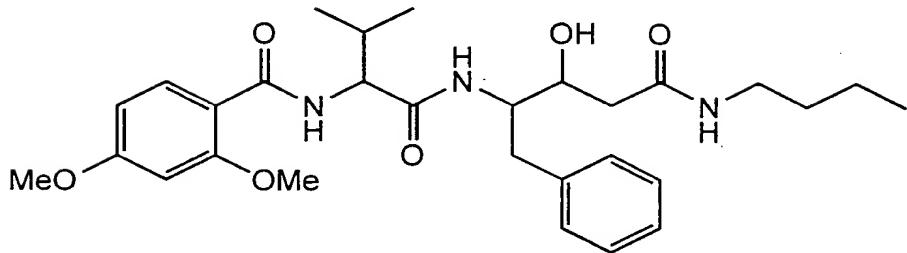
I. Scheme 9

Diamino acid intermediate **23**, one of the diamine ligands (Table 2), is prepared from hydroxy proline **24** by first treating it with alloxychloroformate, in a solvent such as water, in the presence of a base, *e.g.*, potassium carbonate to yield 5 Alloc protected compound **25**. Compound **25** is esterified with either acid in methanol or diazomethane in diethyl ether, producing ester **26**, which is then converted to bromide compound **27** using a brominating reagent such as triphenylphosphine and carbon tetrabromide. The bromide substituent is in turn displaced with azide using either sodium or potassium azide in DMF. Resultant azide compound **28** is then reductively alkylated with acetaldehyde, by first treating it with triphenylphosphine to generate an imine which is in turn reduced to an N-ethyl amino group in compound **29**. Amine **29** is treated with Boc anhydride in acetonitrile to produce compound **30** which in turn is hydrolyzed to carboxylic acid compound **23** by the action of lithium hydroxide in water.

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T250X

EXAMPLE 1
ENTRY 11, TABLE 1



Step 1 - Sequential attachment of bis-Boc lysine, photo-labile linker and an amine

1a. Attachment of bis-Boc lysine to TentaGel™

TentaGel™ resin (S-NH₂, 1.0 g, 0.029 mmol/g, 0.29 mmol, 180-220 μ m) was suspended in a solution of bis-Boc lysine (0.87 mmol, 0.5 g), and HOBr (0.87 mmol, 0.12 g), then treated with DIC (01.7 mmol, 0.27 mL). The suspension was shaken overnight, then drained and washed with 15 mL each DMF (3 x), MeOH (3 x) and DCM (3 x).

1b. Removal of Boc protecting group and attachment of photo-labile linker

A suspension of resin **1** (1.0 g) in 1:1 TFA/DCM was shaken for 1 hour, then washed with 50 mL each DCM (3 x), MeOH (3 x), 4:1 MeOH/Et₃N (1 x), MeOH (3 x), DMF (3 x), then DCM (3 x). This resin was then suspended in 25 mL DCM, then treated with a pre-incubated (45 min) solution of 4-bromomethyl-3-nitro benzoic acid (1.5 mmol, 0.40 g), HOEt (1.5 mmol, 0.23 g), DIC (3.2 mmol, 0.5 mL) in DCM (25 mL). The suspension was shaken for 3 hours, then drained and washed with three 50 mL portions of DCM.

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1c. Addition of amine

One gram of the step 1b resin was suspended in THF (50 mL) and then treated with butylamine (5.4 mmol) and shaken overnight. The resin was then drained and washed with 50 mL each DMF (3 x), MeOH (3 x), 10:1 MeOH/TFA (1 x), MeOH (3 x), DMF (3 x), then DCM (3 x).

5

Step 2 - Addition of phenylalanine-derived statine.

A suspension of the step 1c resin (1.0 g) in DMF (15 mL) was treated with the phenylalanine-derived Boc-protected statine (1.3 mmol), DIEA (2.6 mmol, 0.44 mL), then HATU (1.3 mmol, 0.5 g). This suspension was shaken for 6 hours, drained and washed with 15 mL portions of DMF (3 x), MeOH (3 x), DMF (3 x), and DCM (3 x). The dried resin **9** was divided into two portions.

Step 3 - Deprotection and attachment of Fmoc valine

A suspension of resin **9** (0.5 g) in 40% TFA/ DCM was shaken for 1 hour, then drained and washed with 50 mL each DCM (3 x), MeOH (3 x), 10% Et₃N/MeOH (1 x), MeOH (3 x), and DMF (3 x). The product (0.5 g; 0.33 mmol) was suspended in 10 mL of DMF, containing Fmoc-valine (0.48 mmol) and HATU (0.48 mmol). The suspension was shaken at room temperature for 10 minutes and then DIEA (0.99 mmol) was added. The resulting mixture was shaken for 2 hours, continuously monitoring the resin from the vessel with the Kaiser test to determine the absence of amine functionality. Once the coupling was complete (Kaiser test

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20

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negative), the resin was filtered and washed with 10 mL portions of DMF (3 x), MeOH (3 x) and DCM (3 x).

Step 4 - Fmoc-Deprotection.

The resin (0.5 g) was suspended in 30% piperidine in DMF (15 mL) and shaken for 1 hour at room temperature. The resin was filtered, washed with 15 mL portions of DMF (2 x), DCM (3 x), MeOH (3 x) and DCM (5 x), then dried under vacuum.

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Step 5 - Attachment of 2,4-dimethoxybenzoic acid

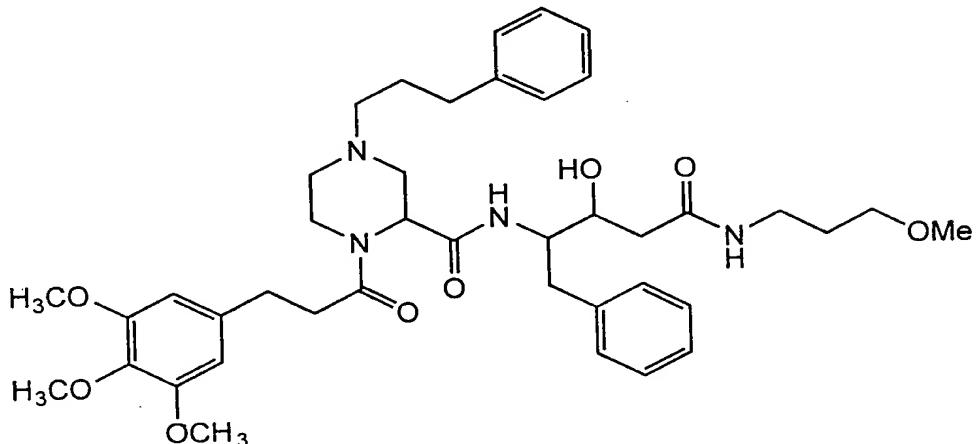
The resin in a reaction vessel was combined with 2,4-dimethoxybenzoic acid (0.6 mmol), HATU (0.72 mmol) and DIEA (1.8 mmol) in DMF (15 mL). The resulting suspension of resin was shaken for approximately one hour at room temperature, at which time the Kaiser test was negative. The resin was filtered and washed with 10 mL each DMF (2 x), MeOH (3 x) and DCM (5 x). The resin was filtered and subjected to a wash cycle consisting of 10 mL portions each TFA/water (1:1) (2 x), DMF (2 x), MeOH (4 x), DMF (2 x) and DCM (5 x), then dried in (15) vacuum.

Step 6 - Cleavage by light

The resin was suspended in MeOH (20 mL) and the compound cleaved from the resin at 50 °C, then light (365 nm) was shone on them for 3 to 4 hours. The

suspension was filtered, the MeOH removed to give the title compound as confirmed by mass spectroscopy (mass spectrum (fab): $m/z = 528$ (MH^+)).

EXAMPLE 2
ENTRY 16, TABLE 2



Step 1- Deprotection and attachment of diamino acid

A suspension of resin **9** (0.5 g) in 40% TFA/DCM, prepared as in Example 1, with the exception that 3-methoxypropylamine was used in place of butylamine, was shaken for 1 hour, then drained and washed with 50 mL portions of DCM (3 x), MeOH (3 x), 10% Et₃N/MeOH (1 x), MeOH (3 x) and DMF (3 x). A suspension of this resin in DMF (50 mL) was treated with the corresponding diamino carboxylic acid (entry 16, Table 2; 1.6 mmol), DIEA (3.3 mmol), then HATU (1.7 mmol). The suspension was shaken for 6 hours, then drained and washed with 50 mL portions of DMF (3 x), MeOH (3 x), DMF (3 x), then DCM (3 x) and filtered. The resin was

dried in *vacuo*.

Step 2 - Deprotection and attachment of a carboxyaldehyde

A suspension of resin batch one (0.5 g) in 40% TFA/DCM (10 mL) was shaken for 1 hour, then drained and washed with 10 mL portions of DCM (3 x), MeOH (3 x), 10% Et₃N/MeOH (1 x), MeOH (3 x), and DMF (3 x). This resin, suspended in 2% HOAc/DMF (10 mL), was treated with 3-phenylpropionyl (8.8 mmol), followed by the addition of NaBH₃CN (4.4 mmol, 0.28 g). The resin was shaken overnight, then drained and washed with 10 mL portions of DMF (3 x), MeOH (3 x), then DMF (3 x), DCM (3 x). The resin was dried in *vacuo*.

Step 3 - Deprotection and attachment of 3-(2,3,4-trimethoxyphenyl)propionic acid

A suspension of resin (0.5 g) in DCM (10 mL) was treated with HOAc (4.8 mmol, 0.27 mL), Pd(PPh₃)₄ (0.072 mmol, 83 mg), then Bu₃SnH (2.4 mmol, 0.64 mL). This suspension was shaken for 1 hour, then drained and washed with 10 mL portions of DCM (3 x), pyridine (3 x), DCM (3 x), then DMF (3 x). The resin in DMF (10 mL) was then treated with 3-(2,3,4-trimethoxyphenyl)propionic acid (0.36 mmol), followed by DIEA (0.72 mmol, 0.13 mL), and HATU (0.36 mmol, 0.14 g). This suspension was shaken for 6 hours, then drained and washed with 10 mL portions of DMF (3 x), MeOH (3 x), then DMF (3 x) and DCM (3 x).

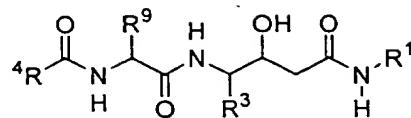
Step 4 - Cleavage by light

The resin was suspended in MeOH (20 mL) and the compound cleaved from the resin at 50 °C, then light (365 nm) was shone on them for 3 to 4 hours. The suspension was filtered, the MeOH removed to give the title compound as confirmed by mass spectroscopy (mass spectrum (fab): m/z = 719 (MH^+)).

5

Using these methods, compounds in Tables 1 and 2 were prepared. The compounds in Tables 1 and 2 typically show greater than 2-fold selectivity for either plasmeprin or cathepsin D at an inhibitory activity (IC₅₀) less than 10 micromolar.

Table 1. R Groups for Compounds of Formula I where Y is an amino acid



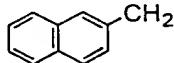
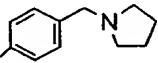
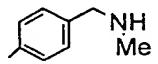
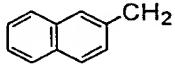
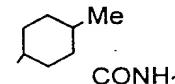
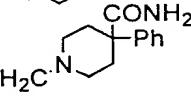
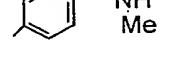
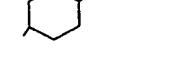
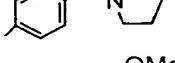
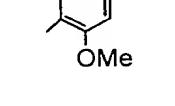
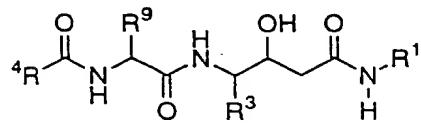
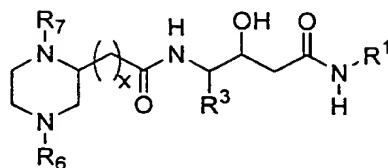
Entry	R ¹	R ³	R ⁹	R ⁴
1	butyl	CH ₂ Ph	CH(Me)CH ₂ Me	
2	butyl	CH ₂ Ph	CH(Me)CH ₂ Me	
3	3-phenylpropyl	CH ₂ Ph	CH(Me)CH ₂ Me	
4	butyl	CH ₂ CH(Me) ₂	CH(Me) ₂	octyl
5	3-phenylpropyl	CH ₂ CH(Me) ₂	CH(Me)CH ₂ Me	
6	3-phenylpropyl	CH ₂ CH(Me) ₂	CH(Me)CH ₂ Me	
7	butyl	CH ₂ Ph	CH(Me) ₂	
8	3-phenylpropyl	CH ₂ Ph	CH(Me) ₂	
9	3-phenylpropyl	CH ₂ Ph	CH(Me)CH ₂ Me	
10	3-phenylpropyl	CH ₂ Ph	CH(Me)CH ₂ Me	
11	butyl	CH ₂ Ph	CH(Me) ₂	

Table 1 (continued). R Groups for Compounds of Formula I where Y is an amino acid



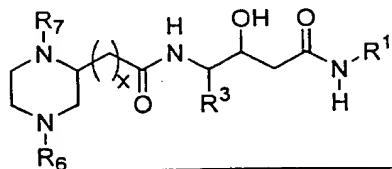
Entry	R ¹	R ³	R ⁹	R ⁴
12	butyl	CH ₂ CH(Me) ₂	Ph	
13	3-methoxypropyl	CH ₂ CH(Me) ₂	CH ₂ SH	
14	butyl	CH ₂ CH(Me) ₂	CH ₂ CH(Me) ₂	
15	butyl	CH ₂ CH(Me) ₂	CH ₂ Ph	
16	butyl	CH ₂ CH(Me) ₂	CH ₂ (2-thienyl)	
17	butyl	CH ₂ Ph	Ph	
18	butyl	CH ₂ CH(Me) ₂	C(Me) ₃	(CH ₂) ₃ -O-(2,4-di-Cl)Ph
19	3-methoxypropyl	CH ₂ Ph	Ph	(CH ₂) ₃ -O-(2,4-di-Cl)Ph
20	butyl	CH ₂ Ph	Ph	
21	butyl	CH ₂ Ph	Ph	(2,4-di-OMe)phenyl

Table 2. R Groups for Compounds of Formula I where Y is the diamino acid

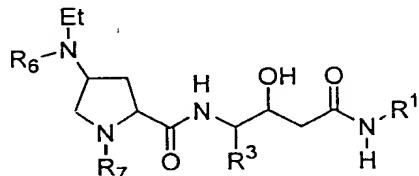


Entry	R ¹	R ³	n	R ⁶	R ⁷
1	benzyl	Me	0	3-pyridinylmethyl	4-isopropoxybenzoyl
2	butyl	CH ₂ Ph	0	Ph(CH ₂) ₂ O(CH ₂) ₂ -	(3,4,5-tri-OMe)benzoyl
3	butyl	CH ₂ Ph	0	4-phenylbenzyl	(3,4,5-tri-OMe)benzoyl
4	butyl	CH ₂ Ph	1	(3,4,5-tri-OMe)benzyl	4-isopropoxybenzoyl
5	butyl	CH ₂ Ph	1	(4-MeC(O)NH)PhCH ₂ -	3-phenoxybenzoyl
6	3-phenylpropyl	CH ₂ Ph	0	4-phenylbutyl	(3,4,5-tri-OMe)benzoyl
7	butyl	CH ₂ Ph	0	(4-MeC(O)NH)PhCH ₂ -	(3,4,5-tri-OMe)benzoyl
8	butyl	CH ₂ Ph	0	3,4-di-Cl-benzyl	(3,4,5-tri-OMe)benzoyl
9	butyl	CH ₂ Ph	0	4-phenylbenzyl	(3,4,5-tri-OMe)benzoyl
10	butyl	CH ₂ Ph	1	(3,4,5-tri-OMe)benzyl	3-phenoxybenzoyl
11	methyl	CH ₂ Ph	0	4-phenylbenzyl	3-phenoxybenzoyl
12	3-methoxypropyl	CH ₂ Ph	1	3,5-bis-trifluoromethylbenzyl	4-isopropoxybenzoyl
13	butyl	CH ₂ Ph	0	3-phenylpropyl	(3,4,5-tri-OMe)benzoyl
14	methyl	CH ₂ Ph	0	4-phenylbenzyl	3-(2-OMe-phenyl)propyl
15	methyl	CH ₂ Ph	0	3,4-di-Cl-benzyl	nicotinoyl

Table 2 (continued). R Groups for Compounds of Formula I where Y is a diamino acid



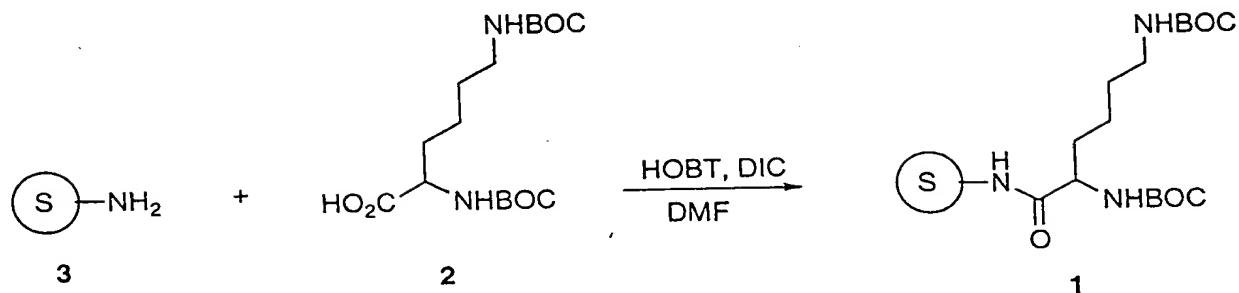
Entry	R ¹	R ³	n	R ⁶	R ⁷
16	3-phenylpropyl	CH ₂ Ph	0	3-phenylpropyl	(3,4,5-tri-OMe-phenyl)propionyl
17	butyl	CH ₂ CH(Me) ₂	0	3-phenylpropionyl	3,3-diphenylpropionyl
18	3-methoxypropyl	CH ₂ Ph	0	4-phenylbenzyl	3,3-diphenylpropionyl
19	butyl	CH ₂ Ph	1	4-phenylbenzyl	3,4-di-Cl-phenylacetyl
20	butyl	CH ₂ Ph	0	(3,4,5-tri-OMe)benzyl	(3,4,5-tri-OMe-phenyl)propionyl
21	methyl	CH ₂ Ph	0	3,4-di-Cl-benzyl	EtOC(O)(CH ₂) ₄ C(O)-
22	butyl	CH ₂ Ph	0	EtOC(O)(CH ₂) ₄ C(O)-	3,4,5-tri-OMe)benzoyl



Entry	R ¹	R ³	R ⁶	R ⁷
23	butyl	CH ₂ Ph	4-phenylbenzyl	phenylacetyl
24	2-pyridinylmethyl	CH ₂ Ph	Me ₂ CHCH ₂ -	3-phenoxybenzoyl
25	3-pyridinylmethyl	CH ₂ CH(Me) ₂	propionyl	3,3-diphenylpropionyl
26	3-pyridinylmethyl	CH ₂ Ph	3,5-di-CF ₃ -phenylacetyl	3,3-diphenylpropionyl

T350X

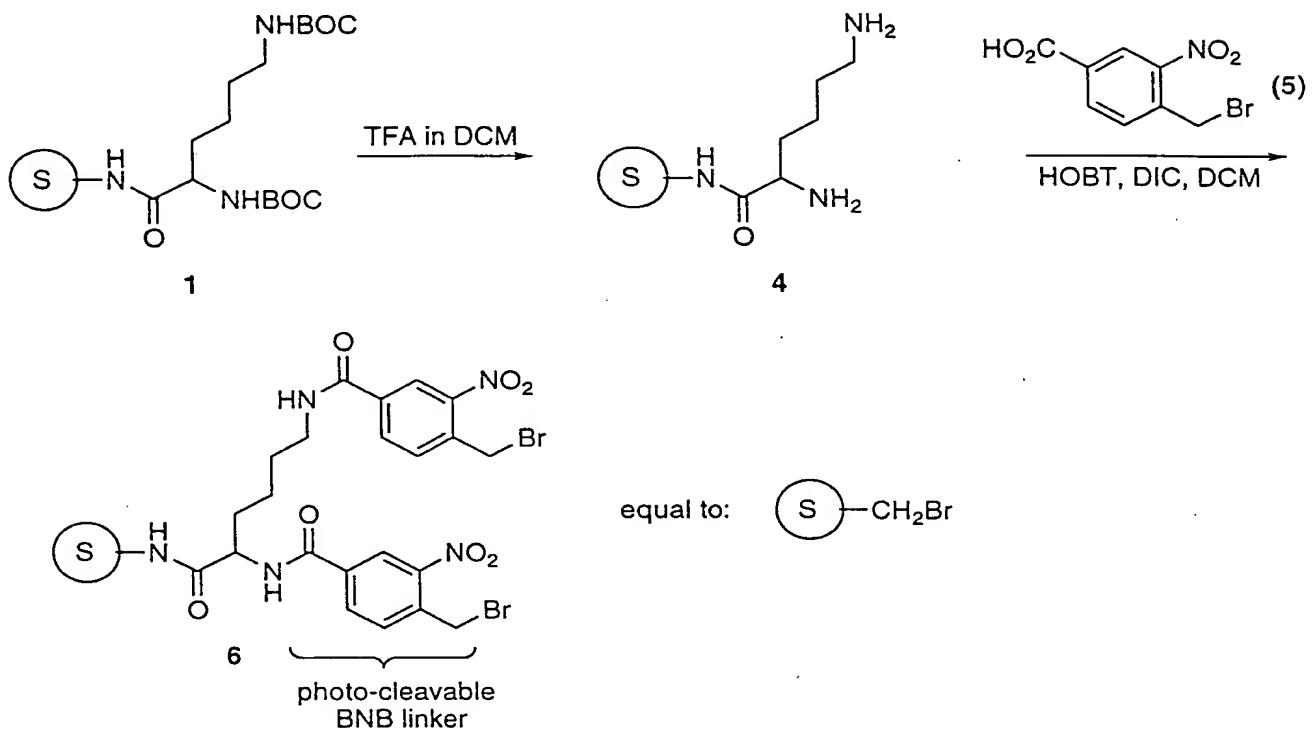
Scheme 1
Attachment of Bis-Boc lysine to resin



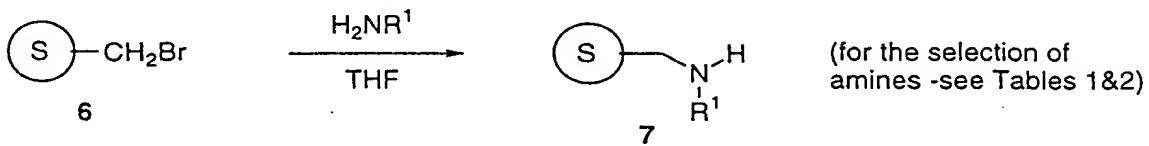
T360X

Scheme 2
Attachment of BNB linker and addition of amine

Step (1): BNB attachment:



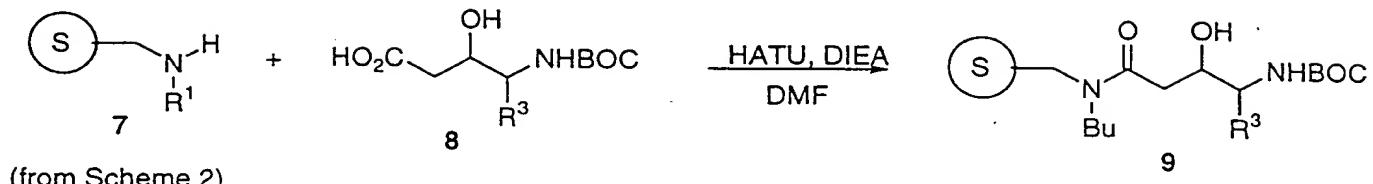
Step (2): Amine attachment:



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T370X

Scheme 3
Attachment of hydroxy-amino acids

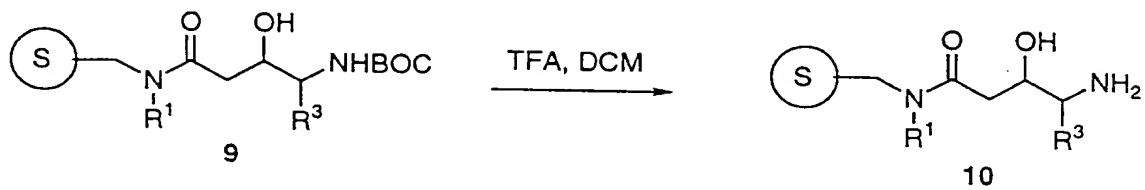


(from Scheme 2)

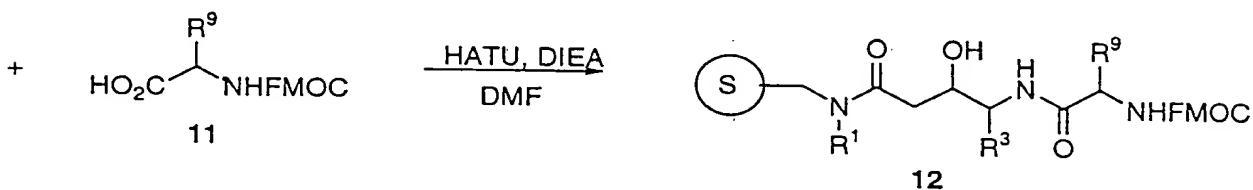
(for the selection of
hydroxy-amino acids
-see Tables 1&2)

T380X

Scheme 4
Removal of BOC-protecting group and attachment of Fmoc-amino acids



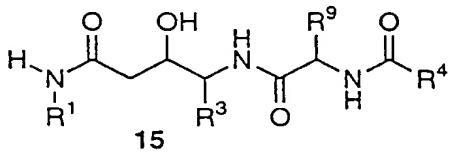
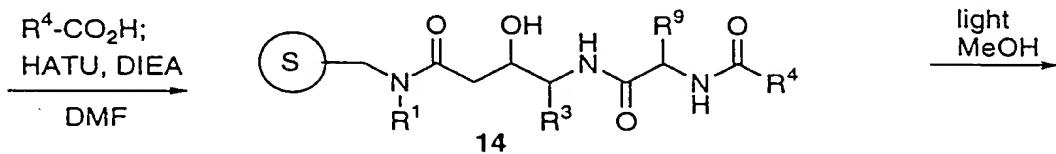
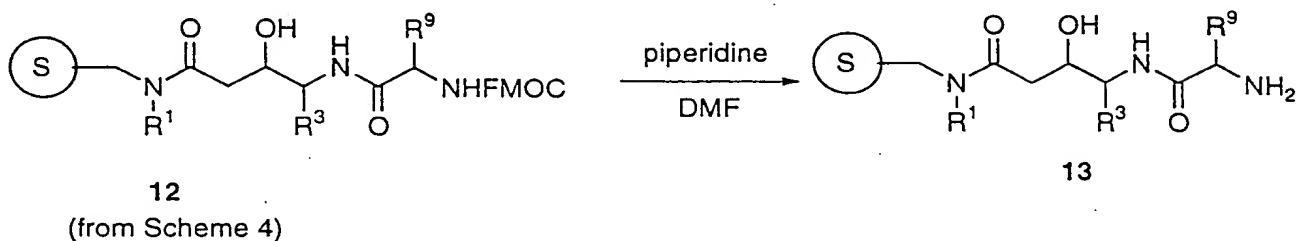
(from Scheme 3)



T390X

Scheme 5

Removal of Fmoc-protecting group, attachment of N-terminal R⁴ groups and cleavage

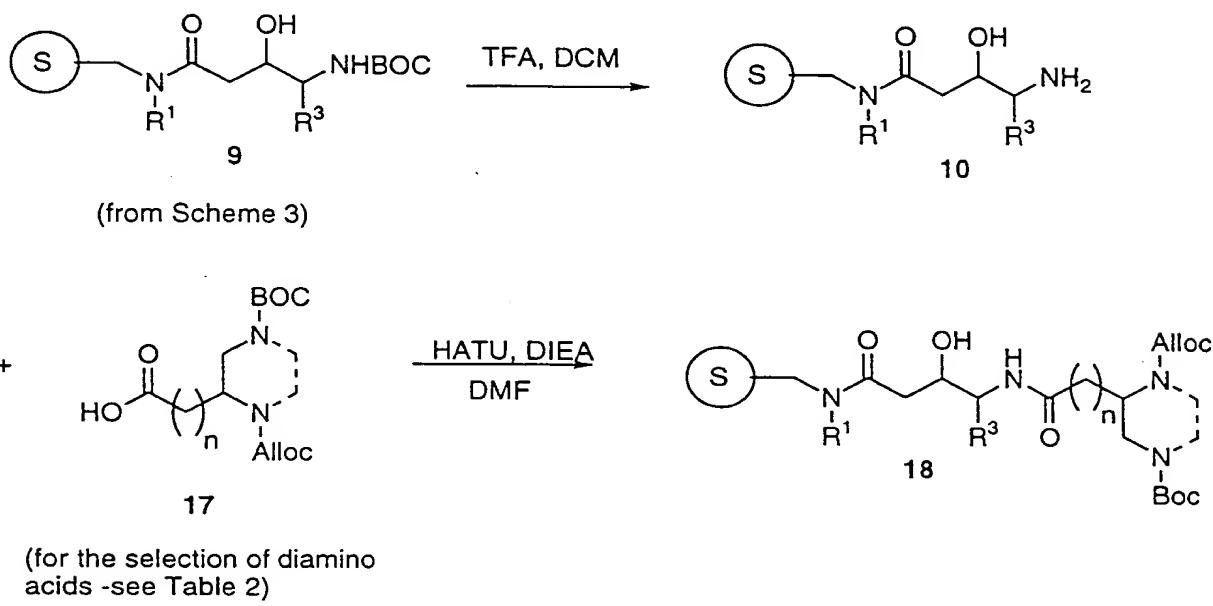


(See Example 1
and compounds in Table 1)

TH00X

Scheme 6

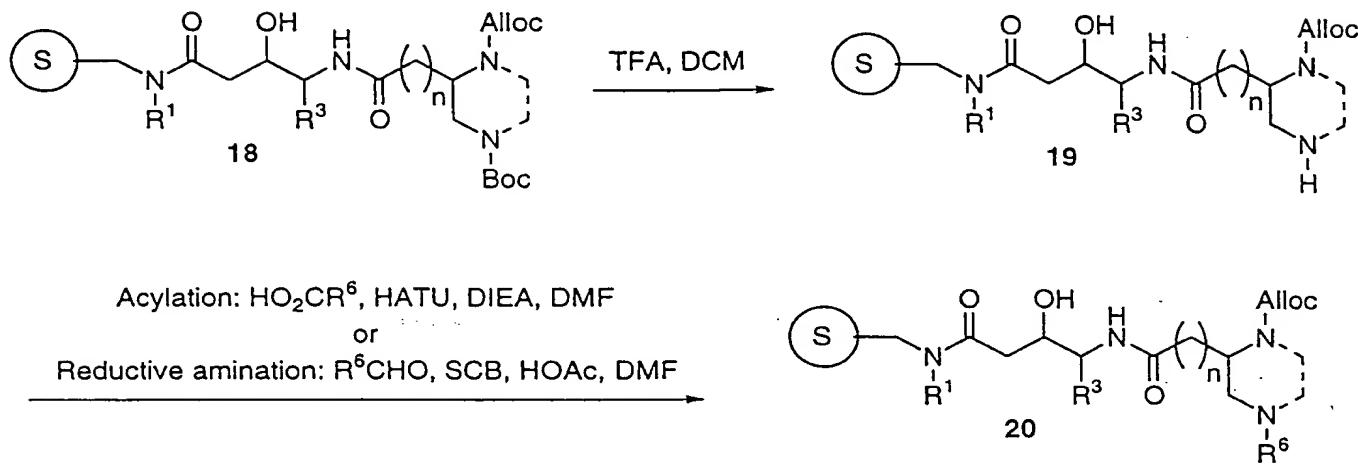
Removal of BOC-protecting group and attachment of diamino acids



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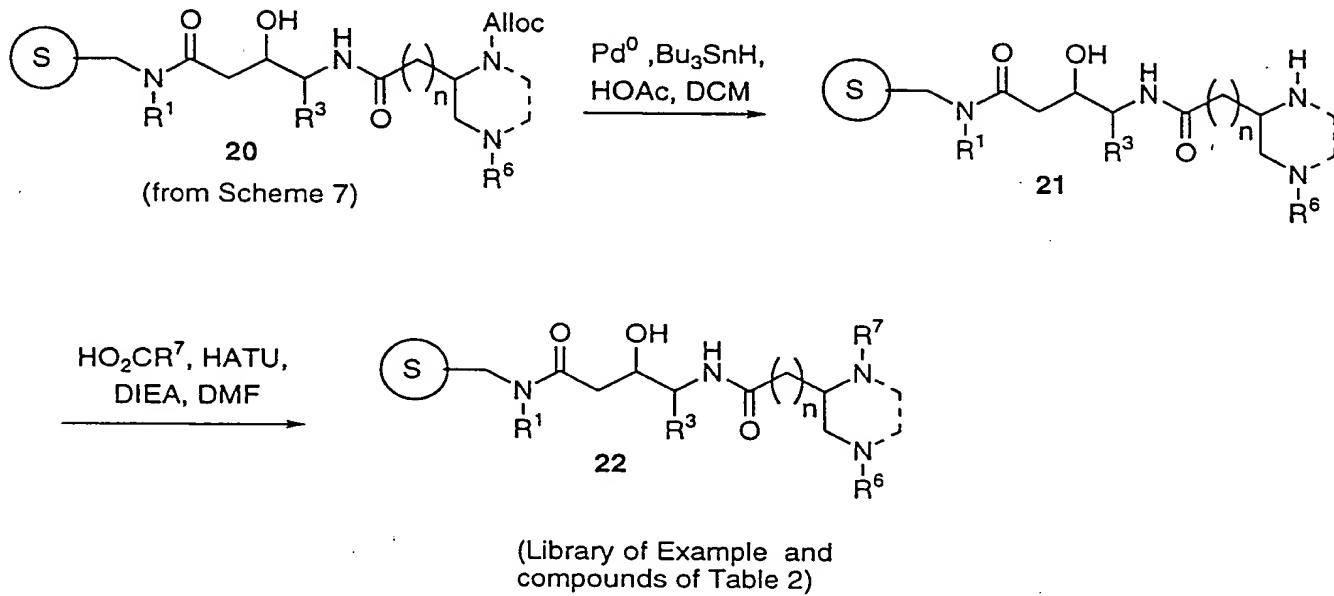
T410X

Scheme 7
Removal of the BOC-protecting group and acylation or
reductive amination of secondary amine



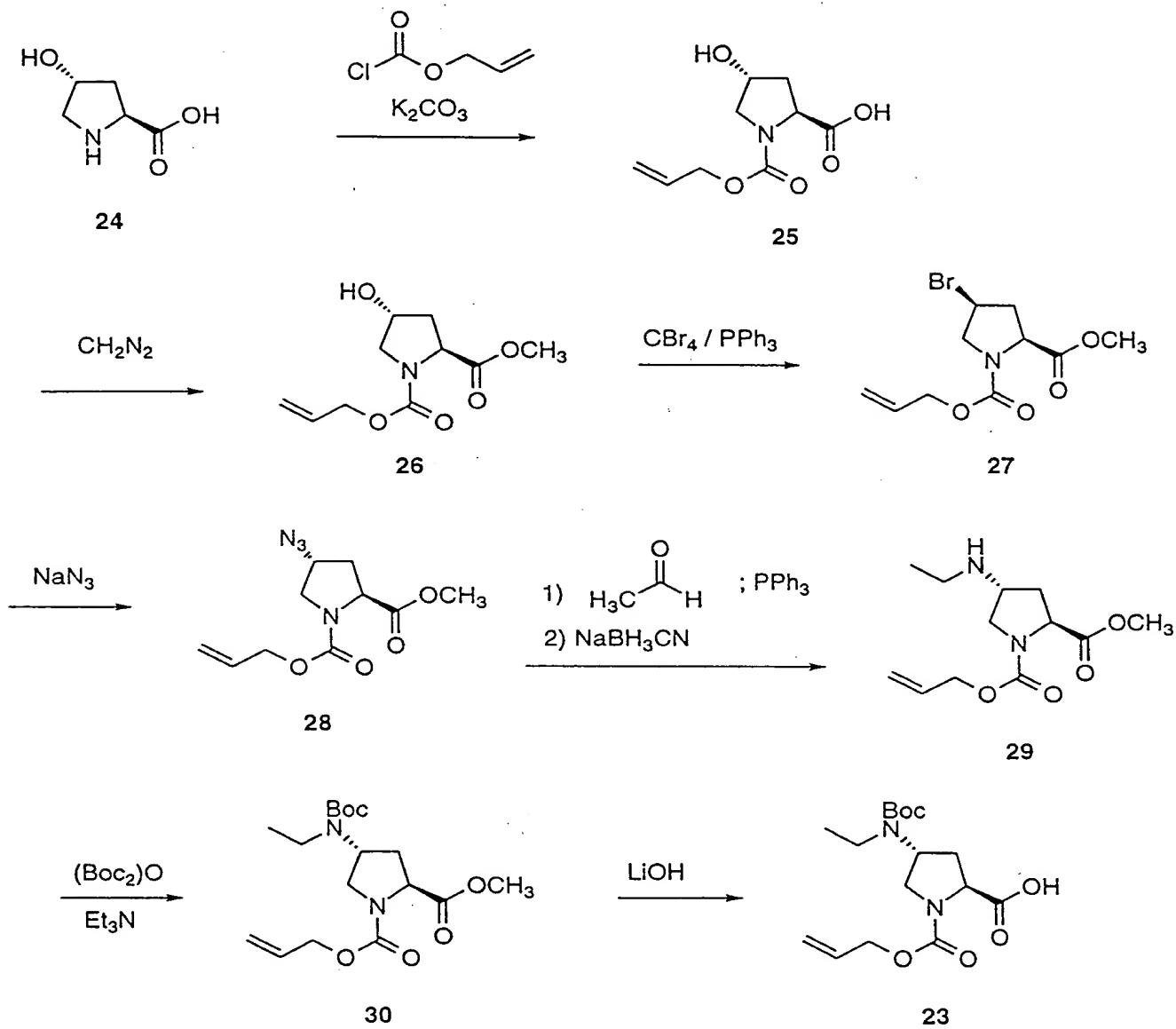
T4Z0X

Scheme 8
Removal of the Alloc-protecting group and acylation of
secondary amine



T430X

Scheme 9
Preparation of Carboxylic Acid 23



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